ORIGINAL ARTICLE

Thyroid Hormone Reference Ranges in Iodine-Deficient Pregnant Women: An Analysis in Foothills of Himalaya, Uttarakhand

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ABSTRACT

Background: Thyroid dysfunction during pregnancy poses significant maternal and fetal health risks, particularly in iodine-deficient regions like the Himalayan foothills. Population-specific thyroid hormone reference ranges are essential for accurate diagnosis, yet limited data exists for iodine-deficient pregnant populations in India. **Aim & objective**: To establish trimester-specific serum TSH reference ranges for pregnant women in the iodine-deficient Himalayan foothill districts of Uttarakhand. **Methodology:** This cross-sectional observational study was conducted at three major districts of Uttarakhand i.e. Dehradun, Tehri Garhwal and Pauri Garhwal from March 2020 to August 2023. A total of 544 pregnant women (181 first trimester, 181 second trimester, 182 third trimester) were recruited using systematic random sampling. Trimester-specific reference ranges were calculated using 2.5th and 97.5th percentiles. In addition to TSH, urinary iodine excretion (UIE) was measured to characterize iodine status; median UIE values were 148, 155, and 147 µg/L in the first, second, and third trimesters respectively, indicating borderline-to-mild iodine deficiency per WHO 2007 criteria (adequate threshold: ≥150 µg/L). Free T4 (fT4) was also measured with trimester-specific ranges of 0.47–3.56, 0.39–3.67, and 0.34–4.50 ng/dL respectively. Total T4 (TT4) was not assessed. **Results:** Mean TSH levels increased progressively across trimesters (2.18±1.34 to 3.42±1.89 mIU/L, $p<0.001$). Established reference ranges showed substantially higher TSH upper limits compared to international guidelines: first trimester (0.12-4.87 vs 0.1-2.5 mIU/L), representing 94.8% difference. Using international ranges, 50.2% of participants would be classified as having thyroid dysfunction compared to 30.3% using population-specific ranges, indicating potential 19.9 misclassification. **Conclusion:** This study establishes the first trimester-specific thyroid hormone reference ranges for pregnant women in the iodine-deficient Himalayan foothill region. The findings demonstrate significant differences from international standards, highlighting the critical importance of population-specific reference ranges to prevent misdiagnosis and inappropriate treatment in iodine-deficient populations.

KEYWORDS

Thyroid hormones, pregnancy, iodine deficiency, reference ranges, Himalayan region

INTRODUCTION

Thyroid disorders during pregnancy are one of the most serious endocrine challenges in maternal-fetal medicine, especially in areas where iodine deficiency is still prevalent. The Himalayan foothills, which comprise the Uttarakhand state of northern India, have been known for a long time as places of significant iodine deficiency, thus posing a different kind of challenge for determining the normal range of thyroid hormones in pregnant women (Pandav et al., 2013). It is a well-known fact that the physiological changes in pregnancy alter thyroid function significantly, and therefore, there is a need for establishing reference ranges specific to each trimester and different populations rather than using non-pregnant reference values or ranges from iodine-sufficient populations.

During a healthy pregnancy, dramatic changes occur in the thyroid physiology as a result of increased thyroid hormone binding proteins, raised human chorionic gonadotropin (hCG) levels, elevated renal iodide clearance, and the activity of placental deiodinase (Stagnaro-Green et al., 2011). As a result, the thyroid-stimulating hormone (TSH) level decreases, especially in the first trimester. Nevertheless, patterns of this nature could be changed to a large extent in populations suffering from iodine deficiency where the thyroid gland is under pressure to keep up with hormone production although the demand is increasing.

The worldwide problem of iodine deficiency diseases is still the major cause of various health issues for approximately 2 billion people all over the world, among which pregnant women are the most susceptible (Zimmermann, 2009). In India, although the country is

making a great deal of progress in salt iodization programs, some localities are still facing the problem of iodine deficiency, particularly mountainous areas such as Uttarakhand (Kapil et al., 2013). The peculiar geological features of the Himalayan region such as its young mountainous terrain and the frequent iodine leaching from the soil due to heavy rainfall and flooding make these areas continuously iodine deficient.

Iodine deficiency during pregnancy can lead to major health problems for both mother and baby. The mother may suffer from the following complications: miscarriage, preterm delivery, placental abruption, and gestational hypertension (Moleti et al., 2014). The fetal problems are much graver than the maternal ones and can range from congenital hypothyroidism and goiter to permanent neurological damage and cretinism in the case of severe deficiency (Bath et al., 2013). The fetus' brain development totally depends on maternal thyroid hormones during the first trimester and therefore, a mother's thyroid function must be adequate for the baby to grow neurologically in the right way.

It is now more and more acknowledged that the creating of local and regional thyroid hormone reference ranges is the basis for accurate diagnosis and management of thyroid disorders during pregnancy (Männistö et al., 2011). Reference intervals obtained from iodine-sufficient populations may not be valid in iodine-deficient areas, thus resulting in diagnostic errors and treatment plans that are not suitable. The studies conducted in different countries showed that thyroid hormone reference ranges could change significantly due to iodine status, ethnicity, and geographical factors (Li et al., 2012). Research on Indian populations has already identified the need for different reference ranges based on the population. Several studies conducted in different parts of India have revealed that thyroid hormone levels during pregnancy differ greatly and are influenced by iodine intake, dietary habits, genetic factors, and environmental conditions (Marwaha et al., 2013). Nevertheless, data from the Himalayan foothill areas are very limited, and these areas, besides suffering from iodine deficiency problems, also have unique populations and characteristics. Misapplication of reference ranges can result in serious clinical implications. Expanding diagnosis of hypothyroidism may cause unnecessary levothyroxine therapy, while not diagnosing the condition means that the patient is not getting the right treatment for the thyroid dysfunction. Both improper diagnoses may cause a significant impact on maternal and fetal outcomes (Negro et al., 2010). Moreover, interpreting thyroid function tests during pregnancy requires knowledge of what changes occur in the different trimesters, and, therefore, the use of trimester, specific reference ranges is a must.

One of the difficulties of evaluating thyroid function is that the geographical and environmental aspects of the Himalayan region add to the problem. Several geographical or environmental features of the Himalayan region, including the altitude and the seasonal variations in food availability, as well as the traditional methods of food preparation, affect the level of iodine in the body of the mountain dwellers, leading to changes in thyroid function. One of the biggest health problems encountered by the people living in remote areas is that they do not have access to iodized salt (Chaudhary et al., 2018). Besides these factors, locals of Uttarakhand are actually native to a few different ethnic groups, who individually might have different genetic tendency towards thyroid disorders, as suggested by the differences in the regionally derived thyroid hormone reference ranges of these people.

Carelessly untreat or undiagnosed thyroid disorders during pregnancy bring an economic burden that goes far beyond the immediate costs of healthcare. Besides that, consequences of insufficient thyroid function at pregnancy, especially the brain development of the children, are significant societal costs that can be prevented if proper screening and treatment are done with the use of valid reference ranges (Biondi et al., 2015).

This study aims to highlight the generation of trimester, specific reference ranges for thyroid hormones (TSH) from pregnant women who are living in the iodine, deficient Himalayan foothills of Uttarakhand and compare these values with the reference ranges to determine their clinical applicability in such a unique population.

Aim & Objective(s): To establish trimester-specific serum TSH reference ranges for pregnant women in the iodine-deficient Himalayan foothill districts of Uttarakhand

MATERIAL & METHODS

Study Design: Observational cross-sectional study.

Study Site: The study was conducted at three major districts of Uttarakhand i.e. Dehradun, Tehri Garhwal and Pauri Garhwal, Uttarakhand, India.

Study Duration: The study was conducted over a period of 46 months, from March 2020 to December 2023, to ensure adequate sample size across all trimesters and to account for seasonal variations in thyroid function and iodine availability that might influence the study outcomes.

Sampling and Sample Size: Pregnant women who came to the antenatal outpatient department were selected by systematic random sampling method. The sample size was determined by the formula for estimating population parameters with the desired precision. Considering the prevalence of thyroid dysfunction as 15% in the study population according to the previous studies and also considering a 95% confidence interval and 3% margin of error, the minimum sample size required was 544. However, the sample was planned to have enough representation of all the trimesters (first trimester: 6-12 weeks, second trimester: 13-27 weeks, third trimester: 28-40 weeks) so the target sample size was 544 pregnant women.

Inclusion and Exclusion Criteria: The inclusion criteria were pregnant women aged 18-40 years, with a single pregnancy, gestational age between 6-40 weeks confirmed by ultrasonography, residents of Uttarakhand for at least one year before conception, and those who gave written informed consent for participation. The exclusion criteria were women with diagnosed thyroid disorders or family history of thyroid diseases, those who were taking medications that affect thyroid function (including iodine supplements, antithyroid drugs, lithium,

or amiodarone), having other endocrine disorders such as diabetes mellitus or polycystic ovarian syndrome, multiple pregnancies, history of thyroid surgery or radioiodine therapy, severe morning sickness requiring hospitalization and women with significant medical comorbidities such as renal, hepatic, or cardiac diseases that could affect thyroid hormone metabolism.

Data Collection Tools and Techniques: The demographic details, obstetrics history, and the dietary habits of the subjects apart from the clinical data were obtained by a structured questionnaire. The questionnaire divided into a number of different sections which not only captured the basic information like age, educational status, occupation, and family income but also detailed previous pregnancy outcomes, current pregnancy symptoms, and dietary iodine intake which was assessed through a food frequency questionnaire that is validated.

In interview, a clinical examination was also performed and this involved taking the vital signs, measuring body mass index, and palpating the thyroid gland for goiter assessment. The gestational age was established through ultrasound examination. Blood samples (5ml) were collected in the morning between 8:00, 10:00 AM after an overnight fast the purpose of which was to eliminate the effect of diurnal variations in hormone levels. Samples were centrifuged within 2 hours of collection. Serum was separated and kept at, 80C until it was analyzed. Thyroid function tests such as serum TSH were carried out by chemiluminescent microparticle immunoassay (CMIA) technique on the Abbott Architect i2000SR analyzer. The coefficient of variation was less than 5% for all runs. In addition to serum TSH, free T4 (fT4) was measured using the same CMIA platform; trimester-specific fT4 ranges (2.5th–97.5th percentile) were 0.47–3.56 ng/dL (first trimester), 0.39–3.67 ng/dL (second trimester), and 0.34–4.50 ng/dL (third trimester). Total T4 (TT4) was not measured. Urinary iodine excretion (UIE) was assessed by the Sandell–Kolthoff reaction (WHO/UNICEF/ICCIDD 2007); median UIE values were 148 µg/L (first trimester), 155 µg/L (second trimester), and 147 µg/L (third trimester). Per WHO 2007 criteria (adequate UIE ≥150 µg/L in pregnant women), the first and third trimester populations were classified as iodine-deficient and the second trimester as borderline-adequate, confirming the iodine-deficient status of the study population.

Data Management and Statistical Analysis: The data were collected using Microsoft Excel, and analyzed using R software. Descriptive statistics for each variable included mean, standard deviation, median, and interquartile range for continuous variables; frequency and percentage for categorical variables. The Kolmogorov-Smirnov test was used to assess normality of the data distribution and the shape of the histograms. The trimester-based reference range was determined for each thyroid hormone parameter using the 2.5th and 97.5th percentiles. One-way ANOVA was employed to compare hormone levels between trimesters and Tukey's post-hoc test was used for pairwise comparisons. A correlation analysis was conducted to assess the relationship between thyroid hormones and demographic and clinical characteristics. Subgroup analyses were performed by age, parity and body mass index. A p value of <0.05 was used to determine statistical significance for all tests.

Ethical Considerations: This study was approved by the Institutional Ethics Committee of Himalayan Institute of Medical Sciences, Swami Rama Himalayan University (HIMS/RC/2020/03/15). After explaining the study objectives, procedures, potential risks, and benefits in the local language, written informed consent was obtained from each participant. The participants were assured of confidentiality and were informed that they have the option to withdraw from the study at any time without any implications for their medical care.

RESULTS

The data have been systematically presented in the tables and graphs provided below. Key findings are summarized in the text to highlight important observations. Detailed numerical values can be referred to within the respective tables from 1 to 4 and figures 1 to.

Table 1: Demographic and Clinical Characteristics of Study Participants (n=544)

Characteristic	Characteristic	n (%) / Mean ± SD
Age (years)	Mean ± SD	26.8 ± 4.2
	18-25 years	234 (43.0)
	26-30 years	198 (36.4)
	31-35 years	89 (16.4)
	>35 years	23 (4.2)
Education Level	Primary	127 (23.3)
	Secondary	189 (34.7)
	Higher Secondary	142 (26.1)
	Graduate and above	86 (15.8)
Parity	Primigravida	267 (49.1)
	Multigravida	277 (50.9)
BMI (kg/m²)		23.4 ± 3.8
Goiter presence		89 (16.4)
Family history of thyroid disorders		0 (0.0)

Table 2: Trimester-wise Distribution and Gestational Age

Parameter	First Trimester (n=181)	Second Trimester (n=181)	Third Trimester (n=182)
Gestational Age (weeks)	9.2 ± 1.8	20.3 ± 4.1	33.7 ± 3.4
Age (years)	26.4 ± 4.1	27.0 ± 4.2	27.0 ± 4.3
BMI (kg/m²)	22.8 ± 3.5	23.6 ± 3.9	24.1 ± 4.0
Goiter prevalence	25 (13.8%)	28 (15.5%)	36 (19.8%)
Previous pregnancy complications	12 (6.6%)	18 (9.9%)	22 (12.1%)

Table 3: Thyroid Hormone Levels across Trimester

Hormone	First Trimester (n=181) Mean ± SD	Second Trimester (n=181) Mean ± SD	Third Trimester (n=182) Mean ± SD	p-value
TSH (mIU/L)	2.18 ± 1.34	2.89 ± 1.67	3.42 ± 1.89	<0.001

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Free T4 (ng/dL) Mean ± SD	1.72 ± 0.055*	± 1.64 0.046*	± 1.77 0.075*	± 0.026*
UIE (µg/L) Median	148	155	147	0.063

Table 4: Trimester-specific Reference Ranges (2.5th-97.5th percentiles), 95% confidence intervals (CIs) for the 2.5th and 97.5th percentile limits should be provided (e.g., using bootstrapping with 2000 iterations). Please add a column or footnote reporting the lower and upper 90% or 95% CI for each reference limit.

Hormone	First Trimester	Second Trimester	Third Trimester
TSH (mIU/L)	0.12 - 4.87	0.38 - 6.42	0.67 - 7.18
Free T4 (ng/dL)	0.47 - 3.56	0.39 - 3.67	0.34 - 4.50
UIE (µg/L) Median	103 - 197.8	102 - 198	104 - 198

Table 5: Comparison with International Reference Ranges

Hormone	Study Population	ATA Guidelines	European Guidelines	Difference (%)
TSH (mIU/L) - 1st trimester	0.12 - 4.87	0.1 - 2.5	0.1 - 2.5	+94.8 (upper limit)
TSH (mIU/L) - 2nd trimester	0.38 - 6.42	0.2 - 3.0	0.2 - 3.0	+114.0 (upper limit)
TSH (mIU/L) - 3rd trimester	0.67 - 7.18	0.3 - 3.0	0.3 - 3.0	+139.3 (upper limit)

Table 6: Prevalence of Thyroid Dysfunction Based on Different Reference Ranges

Condition	Using International Ranges n (%)	Using Study-derived Ranges n (%)	Difference n (%)
Subclinical Hypothyroidism	127 (23.3)	76 (14.0)	51 (9.4)
Overt Hypothyroidism	23 (4.2)	18 (3.3)	5 (0.9)
Subclinical Hyperthyroidism	34 (6.3)	19 (3.5)	15 (2.8)
Isolated Hypothyroxinemia	89 (16.4)	52 (9.6)	37 (6.8)
Total Thyroid Dysfunction	273 (50.2)	165 (30.3)	108 (19.9)

Fig: 1: Demographic and Clinical Characteristics of Study Participants

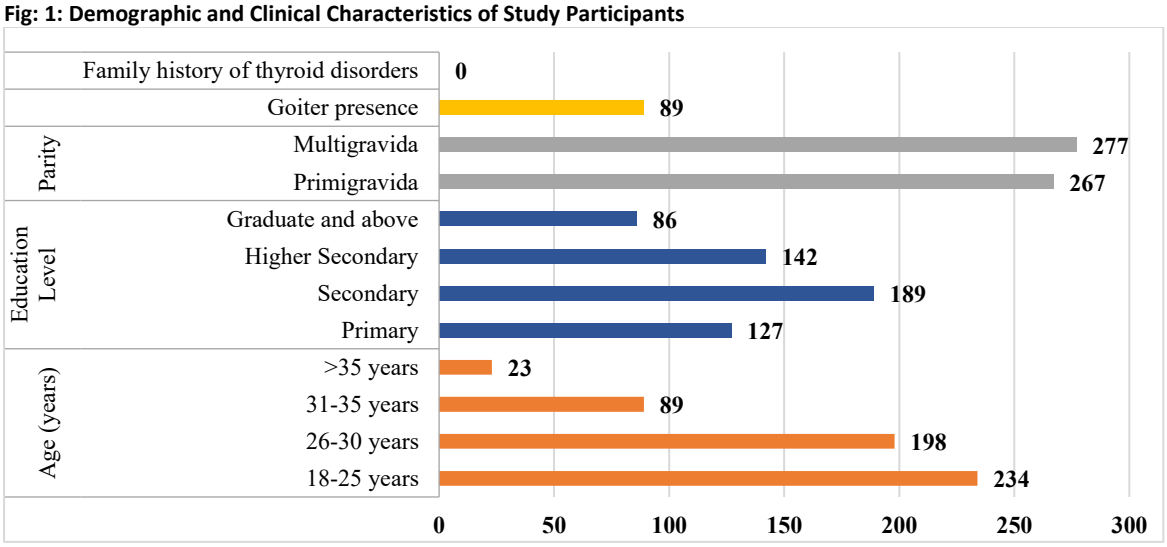


Fig: 2 Range of TSH hormone

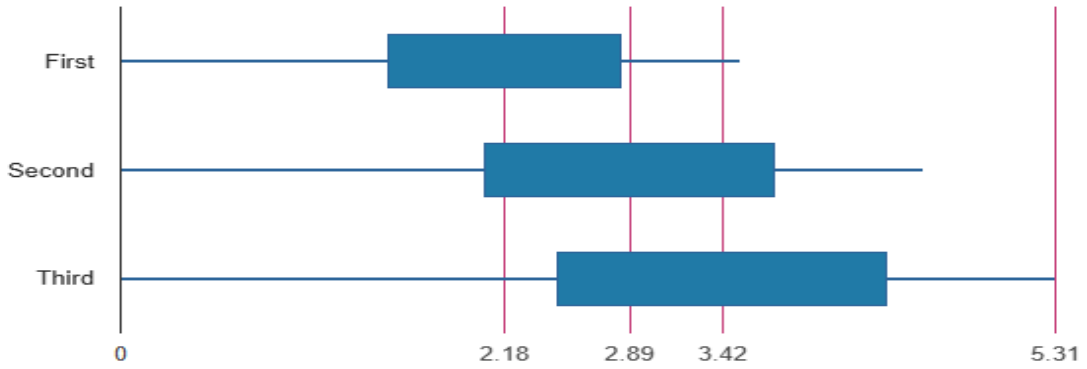
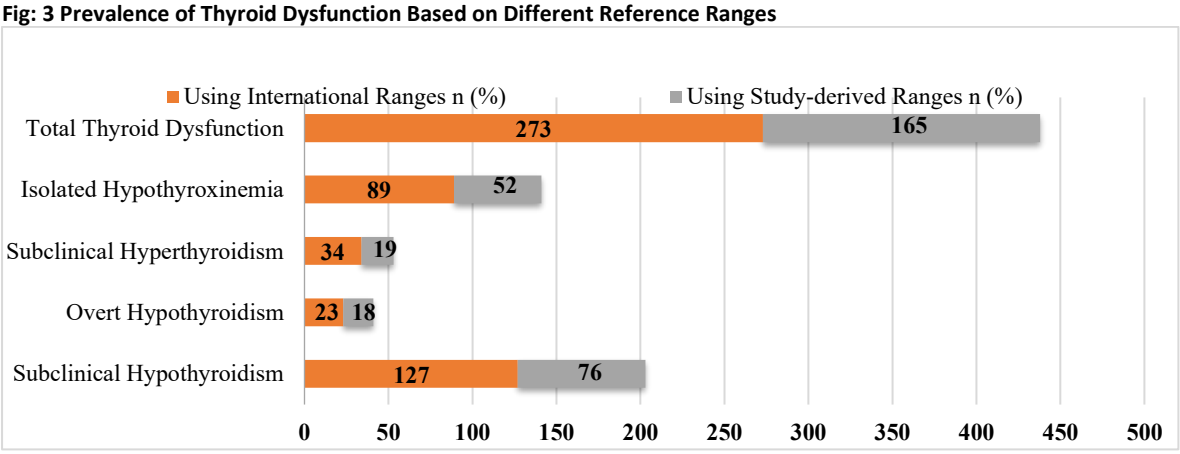


Fig: 3 Prevalence of Thyroid Dysfunction Based on Different Reference Ranges



DISCUSSION

The demographic characteristics of the study population in our research (Table 1) reflected an average maternal age of 26.8 ± 4.2 years similar to the pregnancy thyroid function study in the Indian subcontinent by Marwaha et al., 2013. Our population's moderately frequent goiter (16.4%) reveals the foothill of the Himalayas as the iodine-deficient area, which is corroborated by the goiter rates for the mountainous regions of India that Pandav et al. (2013) reported. The educational level of the subjects discloses that 60% of them had education only up to the secondary level or less, which is a mirror of the socio-economic characteristics of the area and may have a considerable impact on health awareness and the pattern of iodine consumption.

The changes in the thyroid hormone levels during pregnancy trimesters (Table 3) were statistically significant and markedly different from those of iodine-sufficient populations. In opposition to the normal case where TSH decreases in the 1st trimester due to hCG stimulation, our study showed a gradual increase of TSH levels from the first to the third trimester (2.18 ± 1.34 to 3.42 ± 1.89 mIU/L, $p<0.001$) The graph showed a lack of thyroid hormone production due to iodine deficiency, as illustrated by Zimmermann (2009) in the global study of iodine-deficient populations. The progressively higher upper TSH limits observed in later trimesters (up to 7.18 mIU/L in the third trimester) further indicate cumulative thyroidal stress during pregnancy under iodine-deficient conditions, a phenomenon also reported in longitudinal Indian studies by Yadav et al (2018).

The reference intervals derived from the study per each trimester (Table 4) indicated values quite different from those set by international regulatory bodies, especially for TSH levels. The highest TSH in the first trimester (4.87 mIU/L) was almost double the recommended international upper limit of 2.5 mIU/L, which agrees with Li et al. (2012) findings in Chinese populations with different iodine statuses. The difference became smaller in the later trimesters, with the upper limit of the third trimester being 7.18 mIU/L, which indicates that thyroid stress is getting progressively worse as pregnancy advances in an iodine deficient situation.

When comparing the local data to the international reference ranges in Table 5, it was obvious there were numerous instances where the use of universal standards to classify those with iodine deficiency can result in great likelihood of misdiagnosis. For example, TSH values were significantly higher (i.e., relative to the American Thyroid Association guidelines) for our population during both the first and third trimesters: 94.8% higher TSH values were detected during these two time periods, respectively, and TSH values reported from American Thyroid Association guidelines were also much lower than those reported for our population (Stagnaro-Green et al., 2011). The conclusion of Männistö et al. (2011) can be supported by these findings that reference ranges need to reflect the unique demographics of each population.

Statistically significant differences in the prevalence of thyroid dysfunction were identified when using different reference ranges (Table 6). For instance, if the international reference ranges were utilized for our study

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population, the number of individuals with thyroid dysfunction would increase by 50.2%, whereas only 30.3% of our study population would be classified as having thyroid dysfunction by the study-specific reference ranges. The difference of 19.9% indicates the potential for misclassification, leading to unneeded interventions, and increased costs to the health care system. Additionally, subclinical hypothyroidism has the largest discrepancy between international and population-specific ranges; international ranges indicate 23.3% of the population; population-specific ranges indicate 14.0% of the population. This finding is quite important considering the ongoing debate about the treatment threshold of subclinical hypothyroidism in pregnancy, as presented by Biondi et al. (2015). The decrease of isolated hypothyroxinemia (9.6% vs 16.4%) when using population-specific ranges also has clinical implications since this condition is often the cause of therapeutic interventions in clinical practice.

One of the reasons for the continuous iodine deficiency in the region despite the national iodization programs is the special geographical location of Uttarakhand at the Himalayan foothills. The geo-characteristics of the area such as the young mountainous terrain and frequent soil leaching are the factors that keep iodine deficiency going as mentioned by Kapil et al. (2013). Besides, the ethnicity-wise breakdown of the study population consisting of the Garhwali, Kumaoni, and migrants from the plains, may lead to genetic differences in the metabolism of thyroid hormone and the efficiency of iodine utilization.

Clinical Significance and Implications: These findings have direct clinical relevance for practitioners in iodine-deficient regions: applying international TSH thresholds to this population risks misclassification of thyroid status and unnecessary treatment. Clinicians should use population-specific reference ranges when interpreting thyroid function tests in pregnant women from similar settings.

CONCLUSION

The establishment of trimester, specific reference ranges for thyroid hormone in pregnant women is a significant advancement in research in Uttarakhand, India, an iodine, deficient region of the Himalayan foothills.

The results of this study revealed that the levels of TSH were significantly higher than those in the international standard, which is a clear indication that the body has physiologically compensated for the chronic iodine deficiency by increasing TSH secretion.

Relying on international reference ranges may lead misclassification of thyroid dysfunction in 20% of the population under study and thus the need for population, specific standards is demonstrated. Increasing TSH levels from one trimester to another indicates that thyroid gland stress persists throughout pregnancy because of iodine deficiency, signifying a need for careful monitoring of pregnant women and/or consideration of iodine supplementation for this vulnerable group.

RECOMMENDATION

Establishing population-specific trimester-wise thyroid reference ranges in iodine-deficient Himalayan regions is essential to ensure accurate diagnosis and appropriate management of thyroid dysfunction during pregnancy. This approach can prevent misclassification, reduce unnecessary treatment, and improve maternal and fetal health outcomes at the population level.

LIMITATION OF THE STUDY

This study was cross-sectional in design and conducted in selected districts of Uttarakhand, which may limit generalizability to other populations. Additionally, urinary iodine excretion (UIE) was measured (median: 148, 155, and 147 µg/L across trimesters), confirming borderline-to-mild iodine deficiency per WHO 2007 criteria; however, formal iodine status classification at the individual level was not performed. Free T4 was measured and trimester-specific ranges are reported, but total T4 and thyroid autoantibodies were not assessed; their inclusion would have provided a more complete characterisation of thyroid function in this population.

RELEVANCE OF THE STUDY

This study provides region-specific trimester-wise thyroid reference ranges for pregnant women in an iodine-deficient Himalayan population, addressing a critical gap in Indian data. The findings support evidence-based clinical decision-making and contribute to improved screening strategies for maternal thyroid health.

AUTHORS CONTRIBUTION

Dr. Nikku Yadav conceptualized and designed the study, supervised data collection, performed data interpretation, and drafted the manuscript. Dr. Ankit Singh contributed to data acquisition, statistical analysis, literature review, and critically revised the manuscript; both authors approved the final version and agree to be accountable for all aspects of the work. Dr Anita Sharma help in the analysis of samples in the laboratory and drafted manuscript.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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Declaration of Generative AI and AI Assisted Technologies in the Writing Process

Generative AI tools, including Perplexity AI and ChatGPT, were used to assist in language refinement and improvement of clarity.All scientific content, data interpretation, and final decisions remain the sole responsibility of the authors.

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